

## STUDIES ON THE TREMATODE FAMILY MICROPHAL- LIDAE TRAVASSOS, 1921

### IV. THE LIFE CYCLE AND ECOLOGY OF *GYNAECOTYLA NASSICOLA* (CABLE AND HUNNINEN, 1938) YAMAGUTI, 1939

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#### INTRODUCTION

In the first of this series of studies the writer (1939) described *Cornucopula sippavissettensis* gen. et sp. nov. from shore birds near Woods Hole, Mass. Since then, an investigation into the life cycle has shown that *Cercaria nassicola* Cable and Hunninen, 1938, is the larval stage of this trematode. Yamaguti (1939) established a new genus, *Gynaecotyla*, to include species formerly placed in the genus *Levinseniella* but having characters incompatible with those described for *Levinseniella*. Since Yamaguti's paper appeared while the writer's was in press, his generic name has priority; likewise, Cable and Hunninen's specific name has priority; therefore, the correct name of this microphallid trematode is *Gynaecotyla nassicola* (Cable and Hunninen, 1938) Yamaguti, 1939.

Until the brief report by the writer (1939a) of the life cycle of *Cornucopula nassicola* (now *G. nassicola*), no complete life history of a microphallid trematode was known. Numerous reports have indicated larval stages in various genera of this group. Reference to the first three papers of this series (Rankin, 1939, 1939b, 1940) and to the papers of Hadley and Castle (1940) and Cable and Hunninen (1940) will eliminate unnecessary discussion here of the literature on this subject.

The present paper is an elaboration of the original brief report (1939a), presenting an experimental proof of the life cycle of *Gynaecotyla nassicola* with notes on the ecology of this parasite.

It is unfortunate that this genus must again be emended. But since several important characters are either interpreted wrongly or are mentioned as absent when actually they are present, a restatement of the generic diagnosis seems pertinent. In the first place, Yamaguti (1934, 1939) described a female genital sucker lying between the acetabulum and the cirrus or testis; this structure has been shown by the writer (1939) to be a second ventral sucker, with no connection whatsoever

with the genital system. Secondly, Yamaguti indicated that there is no receptaculum seminis or Laurer's canal; actually, these structures are present and can be found even in the metacercaria. Finally, several writers have included this genus in the family Heterophyidae. As will be discussed later, a study of the morphology and life history indicates that *Gynaecotyla* belongs to the family Microphallidae.

*Gynaecotyla* Yamaguti, 1939, char. emend.

Syn.: *Cornucopula* Rankin, 1939

*General Diagnosis*.—Microphallidae Travassos, 1921. Very small, pear-shaped trematodes with spiny cuticula; anterior end bluntly tapering, posterior end broadly rounded. Two ventral suckers located at beginning of posterior body third. Prepharynx, pharynx, and esophagus present; intestinal crura usually extend to posterior level of ventral suckers. Male copulatory organ dextral, consisting of a complicated muscular structure lying in the genital atrium; it is composed of two recurved horn-like projections, the tips of which usually bear spines; pars prostatica weakly developed. Uterus enters genital atrium at lateral edge of dextral acetabulum, close to the common genital opening. Large transverse cirrus pouch, containing bulbous seminal vesicle and coiled ductus ejaculatorius, lies anterior to the ventral suckers. Ovary sinistral, lateral to and on same level as ventral suckers. Testes symmetrical, just posterior to the ventral suckers. Uterine coils filling space behind testes; may extend in front of testes to anterior edge of suckers. Laurer's canal and receptaculum seminis present. Vitellaria follicular, in two compact groups, one behind each testis. Excretory bladder V-shaped; flame-cell pattern, 2 [(2 + 2) + (2 + 2)]. Adults in intestine and caeca of shore birds; metacercariae in crustaceans. Type species: *Gynaecotyla squatarolae* (Yamaguti, 1934) Yamaguti, 1939.

Syn.: *Levinseniella squatarolae* Yamaguti, 1934

*Cornucopula squatarolae* (Yamaguti, 1934) Rankin, 1939

Other species include:

1. *Gynaecotyla jägerskioldi* (Travassos, 1921)

Syn.: *Levinseniella jägerskioldi* Travassos, 1921

*Cornucopula jägerskioldi* (Travassos, 1921) Rankin, 1939

2. *Gynaecotyla simillimus* (Travassos, 1921)

Syn.: *Levinseniella simillimus* Travassos, 1921

*Cornucopula simillimus* (Travassos, 1921) Rankin, 1939

3. *Gynaecotyla adunca* (Linton, 1905)

Syn.: *Distomum aduncum* Linton, 1905

*Levinseniella adunca* (Linton, 1905) Linton, 1928

*Cornucopula adunca* (Linton, 1905) Rankin, 1939

4. *Gynaecotyla nassicola* (Cable and Hunninen, 1938) Yamaguti, 1939

Syn.: *Cornucopula sippivissettensis* Rankin, 1939

*Cornucopula nassicola* (Cable and Hunninen, 1938)  
Rankin, 1939

Figures and descriptions of all five species may be found in the writer's 1939 paper.

#### MATERIALS AND METHODS

Living material was used as much as possible, because many structures, particularly gland ducts and flame cells, cannot be observed in preserved specimens. Neutral red was used successfully as an intravital stain. Material for mounting whole was fixed in Conant's fixative (50 per cent Alc., 100 cc.; Formalin, 6.5 cc.; glacial acetic acid 2.5 cc.), stained in Grenacher's Borax-Carmine precipitated with HCl, and mounted in damar. Material for sectioning was fixed in Bouin's fixative, stained in Delafield's haematoxylin, and counterstained with eosin.

Snails from the infected locality were isolated in fingerbowls to obtain cercariae; crustaceans were examined for metacercariae. In order to obtain parasite-free hosts for experimental infection, snails and crustaceans from widely separated localities were isolated and examined. Young gulls, used as experimental definitive hosts, were taken from the nest when they were four or five days old and kept on a diet of fish and squid.

All drawings were made with the aid of the camera lucida, small details filled in by free hand. All measurements given below are in millimeters.

#### FIELD OBSERVATIONS

While studying the trematode parasites of shore birds in the Woods Hole region, it was observed that these birds were feeding largely, if not entirely, on crustaceans. Stomach contents invariably consisted of digested or partially digested specimens of *Talorchestia*, *Orchestia*, *Gammarus*, etc. The following crustaceans were collected and examined for metacercariae: *Talorchestia longicornis* (Say), *Orchestia platensis* Kröyer, *Chirodotea caeca* (Say), *Gammarus locusta* (Linn.), *Haustorius arenarius* (Slabber), *Crago septemspinosa* (Say), *Palaeomonetes vulgaris* (Say), and *Virbius zostericola* S. L. Smith. In *Talorchestia longicornis* alone were trematode cysts found and these cysts contained metacercariae that appeared morphologically like the adult *Gynaecotyla*

*nassicola* found in the birds. These metacercariae were fed to young gulls and in a few days examination of these experimental hosts produced adult *G. nassicola*, thereby establishing the identity of the metacercariae.

The only snails occurring in abundance in this area are *Littorina littorea* (Linn.) and *Nassa obsoleta* (Say). Thousands of these were isolated. A tiny microphallid cercaria was found in *Nassa*, none in *Littorina*. This was identified as *Cercaria nassicola* described by Cable and Hunninen (1938, 1940). Experimental evidence has yielded positive proof of the identity of this cercaria as the larval stage of *Gynaecotyla nassicola*.

#### EXPERIMENTS PROVING THE LIFE CYCLE

##### *Penetration of Cercaria into Crustacean Host*

Several specimens of *Nassa obsoleta* shedding *Cercaria nassicola* were placed in fingerbowls with *Talorchestia longicornis*, since this was the only species of crustacean found naturally infected. Controls were kept at all times. Examinations were made at regular intervals of both experimental and control animals. Negative results were obtained. Most of the crustaceans were either killed by the snails or drowned. Since this cercaria is the only one of its kind in the locality, the negative results were puzzling. A study of the habits of *Talorchestia* indicated that this amphipod lives in the sand *near* the water line, not *in* the water. In fact, when a fingerbowl had sand in it rising above the water level, the talorchestiae always climbed out of the water on to the sand. This suggested another set of experiments. Three dishes, six inches in diameter and three inches deep, were used. Sand to the depth of one-half inch was placed in each dish with a small mound of it to one side. Water was added to cover the bottom sand, but the mound was left above the water level. Three specimens of *Nassa obsoleta* shedding cercariae were placed in each dish, along with ten *Talorchestia* from an uninfected locality. The water was changed morning and night by running sea water into the sand, thoroughly washing it. This simulated the diurnal changes occurring on the beach. One hundred per cent infection was obtained; the controls proved negative. The first crustacean was examined five days after exposure and contained nine tiny cysts. A corresponding increase in number and size of cysts was found with longer exposure. A maximum of 93 cysts, with an average of 22 per host, was obtained. In nature, an average of 6 cysts, with a maximum of 26, was found in 100 specimens of *Talorchestia* examined. The cysts are found free in the pericardial cavity, not embedded in tissues as is characteristic of other

members of this family (Cable and Hunninen, 1940; Hadley and Castle, 1940).

The method of penetration is quite different from that reported for *Spelotrema nicolli*. In the latter, the crab becomes infected in the water by sweeping the cercariae into the gill chamber where the larvae penetrate into the efferent veins. Since *Talorchestia* is not equipped with this type of respiratory mechanism, a different mode of entrance must be sought. Also, since these amphipods died when left in water alone, another method must be used. Cercariae without tails were found actively moving about in the branchial lamellae (Fig. 1) attached to the second gnathopods and first, second, third, and fourth pereopods. Some were found boring through the tissues towards the dorsal pericardial cavity. These tiny cercariae, then, like so many of the microscopic sand-dwelling species, can live in the moist sand between the tides and can penetrate the amphipods as the latter lie quiescent below the surface. The sand- and mud-burrowing habit of the snail host also aids in completing this stage of the worm's life cycle.

Specimens of *Haustorius arenaria*, *Chirodotca cacca*, *Emerita talpoida*, and *Orchestia platensis* were exposed in a manner similar to that above. Not one of these animals became infected.

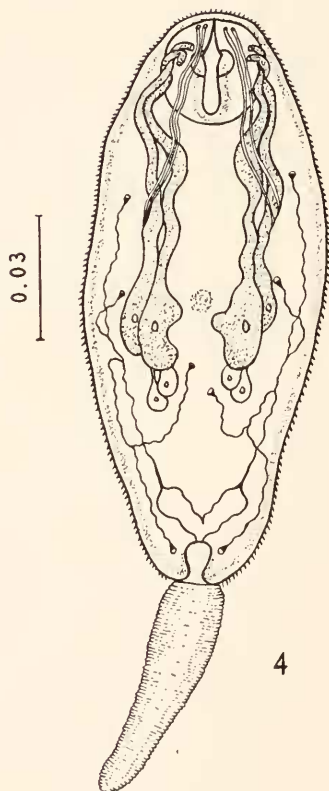
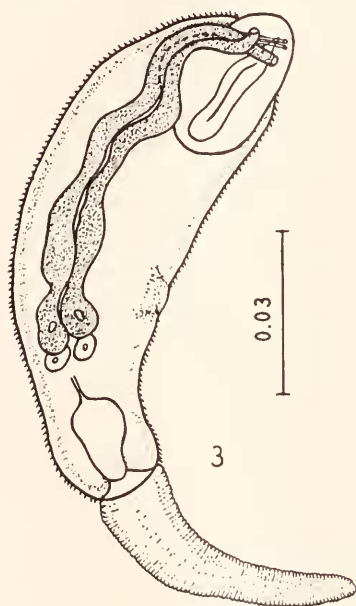
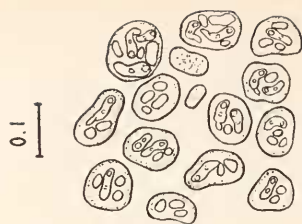
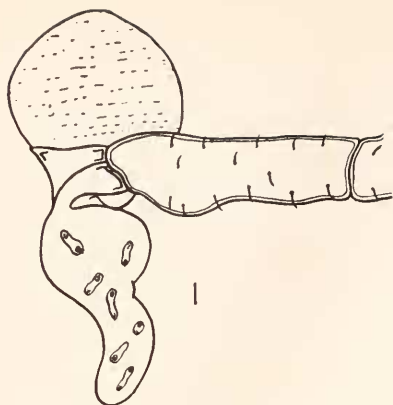
#### *Infection of Definitive Host*

Fifty mature cysts collected from naturally infected talorchestiae were fed to one young herring gull (*Larus argentatus*). Two days later the bird was killed and examined. Twenty-one immature adults of *Gynaecotyla nassicola* were obtained, some with eggs present in the uterus. Sixty-five cysts from experimentally infected talorchestiae were fed to a second gull, and 36 to a third. Thirty-five adults were obtained on examination of the former host seven days later, and 15 from the latter eight days later. Two control birds were negative for this species of trematode.

#### DESCRIPTION OF STAGES IN THE LIFE CYCLE

##### *Sporocyst (Fig. 2)*

The cercariae develop in small oval, thin-walled sporocysts averaging 0.1 in diameter. Many specimens of *Nassa obsoleta* were found to be heavily infected, the livers completely riddled with these larvae. Very few cercariae or germ balls are present, a maximum of 8 cercariae, with an average of 6 being found. A study of sectioned material indicates that two or more sporocyst generations may occur.



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#### EXPLANATION OF PLATE I

FIG. 1. Proximal end of second gnathopod of experimentally infected *Talorchestia longicornis*. Note tail-less cercariae that have penetrated the branchial lamella.

FIG. 2. Cross-section through sporocysts in the liver of *Nassa*.

FIG. 3. Lateral view of cercaria showing openings of glands, unusual thickness of gland ducts, position of stylet, and primordium of ventral suckers.

FIG. 4. Ventral view of cercaria showing arrangement of glands and excretory pattern.

FIG. 5. Stylet of cercaria.



*Cercaria* (Figs. 3, 4, 5)

Since Cable and Huuninen have described this cercaria well, only additions will be given here. *Cercaria nassicola* is a tiny xiphidiocercaria of the ubiquita group, averaging 0.2 in length. It is a slow but continuous swimmer when above the bottom; as soon as it touches the bottom, however, it moves actively over it. Touching the cercaria while it was swimming did not cause cessation of motion. No ventral sucker as such has been observed. In sectioned specimens, however, a small vacuole or cavity may be found in the region of the future suckers. Likewise, in some flattened specimens, a thickening in this region may be found. The number of glands and arrangement of ducts were found to be as described. Four flame cells were found on each side, in the formula  $2[(1 + 1) + (1 + 1)]$ . The stylet is quite large in comparison with the size of the oral sucker, measuring 0.023 in length. It is lancet-shaped anteriorly, bluntly rounded posteriorly, while an elongate keel protrudes a short distance ventrally.

*Metacercaria* (Figs. 6, 7)

The metacercariae occur free in the pericardial cavity of the crustacean. These roll out when the host is torn open, with no host tissue attached. In the smaller cysts, the stylet remains in position. As the metacercaria grows, the stylet is absorbed, not shed, for it was never found either in position or in the cyst cavity in the larger metacercariae. Remains of stylet glands were present in all larvae found. Most of the adult characters were found even in the youngest metacercariae examined: the two acetabula, the genitalia, and the convoluted walls of the intestinal crura. The cysts averaged 0.26 in diameter, never exceeding 0.29. The cyst wall averaged 0.015 in thickness. The body is covered with spines as far posteriorly as the acetabula. Large refractile glands are located beneath the cuticula of the whole body and render difficult observation of body systems, particularly the excretory system. The excretory bladder appears to be without a lumen, filled with large cells. The flame cell pattern is the same as that for the adult,  $2[(2 + 2) + (2 + 2)]$ .

The younger metacercariae are removed easily from the cyst but die very quickly. Older specimens, however, are removed with difficulty due to increased thickness of the cyst wall. These may be mounted in mammalian Ringer's solution and remain alive for several hours.

*Adult (Figs. 8, 9)*

Since the adult of this species has been adequately described in a previous paper (Rankin, 1939) only a few controversial details will be discussed. Yamaguti (1934, 1940) considered the second ventral sucker to be a female genital sucker located at the external opening of the uterus. In the present study, however, metacercariae of various ages, ranging from stages with only poorly developed to those with fully developed suckers, have been examined both alive and sectioned. In no case was any connection ever found between the uterus and this second sucker. In development and histological appearance it has every character of a true sucker. Figure 9 indicates the appearance of the two ventral suckers and their striking similarity is readily apparent. Perhaps phylogenetically this second sucker may have been derived from a genital sucker, having lost this former function and connection secondarily. However, the present evidence does not permit such a conclusion, but does indicate the acetabular nature of this structure.

A receptaculum seminis and Laurer's canal have been reported by Yamaguti and others as absent from genera considered here as in the family Microphallidae. The writer (1939, etc.), Cable and Hunninen (1940), and Hadley and Castle (1940) have shown that these structures do exist in species of *Levinseniella*, *Spelotrema*, *Maritrema*, and *Gynaecotyla*. A careful study of live material will probably show these structures to occur in other genera likewise, for only on living specimens have they been observed. They are so thin-walled that they collapse immediately on fixation. In *G. nassicola*, the receptaculum seminis and Laurer's canal may be discerned particularly well in immature specimens in which the uterine coils are not yet filled with eggs (Fig. 8).

*The Egg and Miracidium (Fig. 10)*

The structure and development of the miracidium are very difficult to follow due to its small size. The eggs measure  $0.02 \times 0.01$  and are very numerous. In young worms the eggs are practically undeveloped;

## EXPLANATION OF PLATE II

FIG. 6. Metacercaria within cyst. Note that even through the cyst wall the two acetabula may be observed.

FIG. 7. Metacercaria removed from cyst wall. Stylet still present along with remains of the cephalic or stylet glands; genitalia fairly well developed; flame cell pattern shown.

FIG. 8. Immature adult recovered from an experimentally infected gull. Remains of gland ducts still present. Note especially the two large, similar acetabula, and the large bulbous receptaculum seminis with a short Laurer's canal.

FIG. 9. Cross-section through the ventral suckers and the copulatory complex. The identities of the two suckers are easily discerned.

FIG. 10. Immature egg.



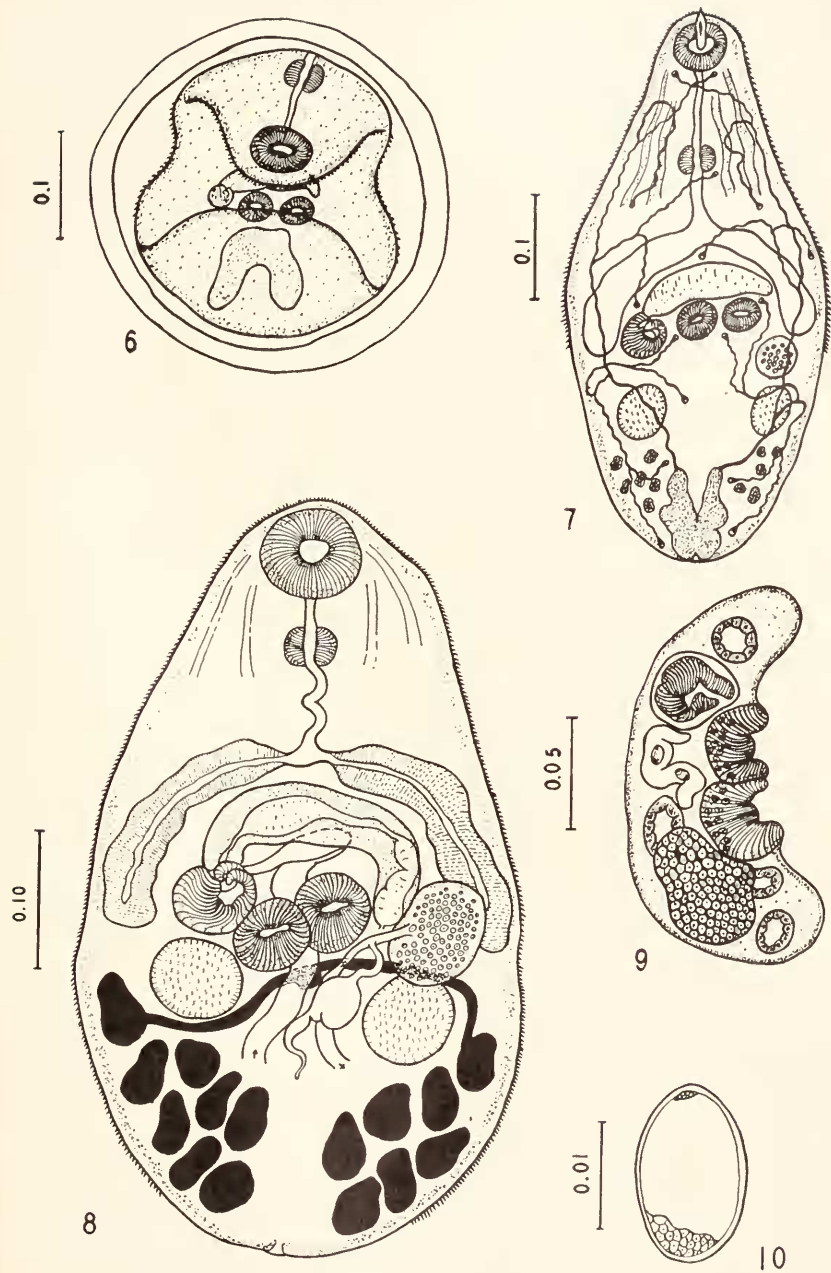


PLATE II

in older specimens, crowding prevents clear observation. Development in sea water or in mammalian Ringer's solution is very slow and no larva has been carried through to hatching. Consequently, the mode of penetration into the snail host has not been observed.

#### ECOLOGY OF *GYNAECOTYLA NASSICOLA*

The relationship between the habits of host and parasite is well illustrated in the life cycle of *Gynaecotyla nassicola*. In a comparatively small area all three hosts occur in relative abundance. *Nassa obsoleta* literally covers the beach at the infected locality near the low tide zone. As the water recedes at low tide, the snails are exposed and crawl about on the sand or burrow beneath the surface. The crustaceans, likewise, emerge by the thousand from the sand at low tide and have been seen running across the damp surface in hordes. Plovers, sandpipers, and other shore birds may be observed running up and down the beach feeding on these amphipods. Other species of crustaceans usually remain buried in the sand or under drift weed along the water's edge. *Talorchestia* is the only one exposed so plentifully to the birds.

A study of the seasonal distribution of infection of *Nassa* with the cercariae of *G. nassicola* yielded the following results (the first figure after the date is the number of snails examined; the second figure is the percentage of infection): July, 1939: 717, 2.9; August: 481, 0; September: 815, 0; March, 1940: 525, 9.2; April: 576, 21.9; May: 611, 34.8; June: 1570, 6.1; July: 2640, 0.01; August: 2000, 0; September: 1250, 0. Examination of these figures indicates a relatively high snail infection in spring and little or no infection in fall and winter. A similar study of *Talorchestia* indicates little infection with metacercariae in early spring, but an increase to a maximum in late July and August.

These results might be interpreted as follows: When the birds arrive early in February and March during their northward migratory period, eggs of the parasite are dropped in the feces along the beach as the birds feed at low tide. Miracidia hatch, penetrate *Nassa obsoleta*, and develop into sporocysts. The birds stay at Woods Hole only for a few days at this time and then resume their northward trek to the breeding grounds. When they return in the fall, the cercariae have matured, penetrated the beach fleas, and developed into ripe metacercariae. The birds, then, may become reinfected and carry the worms back on their southward migration. Possibly the snails might become reinfected at this time also, the developing larvae remaining dormant through the winter. But since little or no infection was found in early spring, this hypothesis does not seem well grounded.

## DISCUSSION AND CONCLUSIONS

Until the life cycles of *Gynaecotyla nassicola*, *Maritrema arenaria* (Hadley and Castle, 1940), and *Spelotrema nicolli* (Cable and Hunninen, 1940) were described, an understanding of the relationships of the Microphallidae was handicapped considerably. The genera of this family were included with the Heterophyidae on purely superficial morphological characters. Ward (1901) erected the subfamily Microphallinae to contain these genera, but he still retained them under the Heterophyidae. Travassos (1921) raised the Microphallinae to family rank, excluding it from the Heterophyidae, as did Viana (1924). Witenberg (1929) likewise excluded the Microphallinae from the Heterophyidae. In spite of these observations, various investigators have retained the microphallids under the Heterophyidae. It seems pertinent at this point, therefore, to indicate briefly the main differences between these two families of trematodes. In the Microphallidae, the uterus lies either wholly posterior to or overlapping the testes; there is a well-developed acetabulum, not enclosed with the genital suckers; the genital pore is not closely associated with the acetabulum; the excretory system is always of the formula  $2[(2 + 2) + (2 + 2)]$ ; and the life cycle always includes a crustacean as the second intermediate host. In the Heterophyidae, the uterus lies anterior to the testes; the acetabulum is usually closely associated with the genital structures; the excretory system is never of the above pattern, but consists of large numbers of flame cells on each side of the body; and the life cycle usually includes a fish as the second intermediate host. In the first family, a crustacean-eating animal (usually a bird) is the definitive host, whereas in the second family a fish-eating animal (mammal or bird) is the definitive host. These characters, along with other morphological features, render impossible the inclusion of members of one with those of the other. As pointed out by the writer (1939c) and Cable and Hunninen (1940), the complete life cycle of a species should be known before final conclusions can be drawn with respect to relationships, distribution, etc.

The infection of second intermediate hosts through the respiratory system by both *Gynaecotyla nassicola* and *Spelotrema nicolli* would indicate that other members of this family infect the hosts through this system rather than through the digestive tract. Depending on the type of respiratory apparatus of the particular crustacean, the cercariae are either swept in passively through a gill chamber and then penetrate, or actively bore into branchial lamellae.

Considering the results obtained from a seasonal-distribution study of the northward range of the cycle, it would prove of considerable in-

terest to examine the different hosts at the southern range. A similar distribution might occur. If this were so, then added evidence for the hypothesis that the northern snails do not remain infected during the winter would be obtained.

A specificity for hosts is evidenced to a marked degree by the different stages of *G. nassicola*. *Nassa obsoleta* and *Talorchestia longicornis* are the only intermediate hosts found infected or infectable, of the many examined. The distribution of these hosts in the vicinity of the definitive host may account for this phenomenon. Should the habits of other mollusks and crustaceans bring them in proximity to the birds, then they in turn might be expected to become infected. Yet, since no other species could be experimentally infected, the ecological factor is not the determining one. Some physiological difference seems evident. Host preference has been reported by various writers (Lühe, 1909; Dubois, 1929; Wesenberg-Lund, 1934; Rankin, 1939c; etc.) and seems to be a fairly common phenomenon.

#### SUMMARY

The life cycle of the microphallid trematode, *Gynaccotyla nassicola* (Cable and Hunninen, 1938) Yamaguti, 1939, has been determined experimentally and the various stages described and figured. The miricidia penetrate the mud snail, *Nassa obsoleta* (Say), develop into oval sporocysts that produce daughter sporocysts and cercariae of the ubiquita type. The cercariae penetrate the branchial lamellae of the sand flea, *Talorchestia longicornis* (Say), then migrate through the tissues to the pericardial cavity where they encyst. Metacercariae develop into adults when the crustaceans are eaten by shore birds (plovers, sandpipers, etc.) or fed to experimental hosts (gulls).

The ecology of the hosts and parasite is discussed, indicating the close relationship between the distribution of the various animals involved. The differences between the Heterophyidae and the Microphallidae are indicated.

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